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Hormonal and Gravitropic Specificity in the Regulation of Growth and Cell Wall Synthesis in Pulvini and Internodes from Shoots of *Avena sativa* L. (Oat)

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Segments can be cut from the peduncular-1 internode of oat (*Avena sativa* L.) shoots so as to contain the graviresponsive leaf-sheath pulvinus and gibberellin-sensitive internodal tissue. Incorporation of [^{14}C]glucose was used to monitor cell wall synthesis in these two tissues as affected by gravistimulus, indoleacetic acid (IAA), gibberellic acid (GA_3), and fusicoccin (FC). Pulvinar cell wall synthesis was promoted by IAA and FC (both within about 1 h), as well as by gravistimulus (starting between 3 and 6 h), whereas GA_3 had no effect on nongravistimulated pulvini. In contrast, GA_3 and FC promoted internodal cell wall synthesis (initiated between 1 and 2 h), whereas IAA and gravistimulus caused a decrease in internodal uptake. FC preferentially promoted incorporation into the matrix component of the wall in both tissues. Gravistimulus failed to increase responsiveness of pulvinar tissue to IAA, whereas GA_3 partially overcame gravistimulus-promoted incorporation into pulvinar cell wall, probably because of preferential movement of label into the rapidly elongating internode. The results demonstrate that these eight stimulus/tissue combinations can be examined easily in an isolated 10-mm stem segment, providing new opportunities for the comparative study of tissue- and stimulus-specific events in gene regulation and signal transduction in agronomically important cereals.

Segments cut from the next-to-last (peduncular-1) internode of 45-d-old oat (*Avena sativa* L.) plants so as to contain the intercalary meristem undoubtedly constitute one of the most useful systems available for the study of GA-induced elongation. The internodal tissue (encircled by the leaf sheath) within these 10-mm segments responds with specificity and high sensitivity to the hormone, elongating to as much as 80 or 90 mm in 80 h at 30°C when supplied with 0.1 M Glc (Montague, 1993, and refs. therein). This growth response is highly specific for GA in that it is inhibited by auxin (Kaufman et al., 1969), cytokinin (Jones and Kaufman 1971), and ABA (Kaufman and Jones, 1974), as well as by ethylene and jasmonic acid (M.J. Montague, unpublished data). GA_3-promoted elongation is accompanied by enhanced cell wall extensibility (Adams et al., 1975) and increased wall synthesis from exogenous [^{14}C]Glc (Montague and Ikuma, 1975, 1978); the latter, initiated about 1 h after hormone application, is among the earliest demonstrated biochemical responses elicited by GA in any system.

The segments also contain the graviresponsive leaf-sheath pulvinus (Brock and Kaufman, 1990), an anatomical feature of several important cereals (e.g. oat, barley, wheat, rice). Given an appropriate gravitational stimulus (e.g. through lodging), pulvinar cells begin to elongate differentially within 0.5 to 1 h, ultimately causing the shoot to return to a more upright orientation (Kaufman and Dayanandan, 1985). This graviresponse seems to be mediated primarily either by an increase in free auxin levels (Kaufman et al., 1987) or by altered responsiveness to auxin, apparently without the participation of cell division (Dayanandan et al., 1976; Brock and Kaufman, 1990). Although GA appears to play some role in modulating the response to gravistimulus (Pharis et al., 1981; Brock and Kaufman, 1988a), it has no effect on pulvini from vertical segments. As with GA_3-promoted internodal elongation, gravistimulated pulvinar growth is accompanied by enhanced cell wall extensibility (Gibeaut et al., 1990).

Auxin-stimulated cell elongation in other systems is commonly accompanied by increased synthesis of cell wall as a direct effect of the hormone (Baker and Ray, 1965; Abdul-Baki and Ray, 1971; Brummell and Hall, 1985; Bret-Harte et al., 1991), and enhanced cell wall synthesis is clearly correlated with GA_3-mediated elongation of the *Avena* internode (as noted above). However, data concerning the involvement of cell wall synthesis in gravistimulated or auxin-induced growth of the leaf-sheath pulvinus have been equivocal or absent (for contradictory results, see Gibeaut et al., 1990; Lu et al., 1992).

The primary purpose of the present investigation was to determine whether gravistimulation and/or auxin (IAA) in fact acted to promote cell wall synthesis from exogenous carbohydrate in the pulvini of *Avena* stem segments and, if so, to characterize these responses and their kinetics. The second objective was to use FC (as a nonphysiological stimulus) with kinetics analysis to compare GA_3-mediated effects in the internode with IAA-mediated effects in the pulvinus. Ultimately, the goal of this work was to understand more completely the relative roles, specificities, interactions, and possible causal interrelationships of gravistimulation, auxin, and GA in the developmental physiology of shoots from this agronomically important group of grasses.

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Abbreviation: FC, fusicoccin.

MATERIALS AND METHODS

Plant Material

Oat plants (*Avena sativa* L. cv Victory; seed graciously supplied by Svalöf AB International, Svalöv, Sweden) were grown in 6-inch pots (200 seeds per pot) in a growth chamber (16 h of light/8 h of dark; 18/16°C) with a light intensity of 800 μE. Shoots were collected after about 45 d of growth. (For convenience, collected shoots were routinely held at 4°C for several days until further preparation.) Stem segments were ordinarily prepared from shoots that contained the internode immediately below the peduncular node (p-1 internode) with a length between approximately 10 and 40 mm, which ensured maximum growth response to GA_3 (Adams et al., 1973), as well as adequate gravitropic response. This developmental staging was a compromise between maximum internodal elongation potential (Adams et al., 1973), high pulvinar graviresponsiveness (Brock and Kaufman, 1988b), and reduction in segment variability.

Tissue Preparation and Growth Conditions

Segments (10 mm) containing the node, pulvinus, and leaf sheath, together with the internode, were prepared with a razor blade cutting device. Such segments were designated whole segments because they contained all tissues. After treatment with various stimuli, internodal tissue was dissected from the pulvinus, node, and leaf sheath so that each of the tissues could be collected and analyzed separately. This dissection was accomplished by using a razor blade first to remove the node from the segment, then to cut through the pulvinus to free the internodal tissue, and finally to sever the other half of the pulvinus from the remaining leaf sheath. For some experiments, 10-mm segments were prepared with the same cutting device but in a manner in which the connection between the internode and node was severed so that the internodal tissue was easily dissected from the pulvinus, node, and leaf-sheath portions. Such segments were designated isolated internodes because they contained only the internodal tissue (10 mm).

Whole segments or isolated internodes were placed upright in perforated plastic or plexiglass frames on filter paper in the lids of 55-mm plastic Petri dishes. Each dish contained 1 to 3 mL of treatment solution, which was taken up from the base of each section. Thus, the existing vasculature delivered nutrient and hormone to the segment. GA_3 was usually supplied at 0.5 mM, a concentration well in excess of the minimum necessary for maximum growth to ensure that hormone was never rate limiting. D-[U-^{14}C]Glc (specific activities noted in figure legends) was supplied at 0.1 M. This concentration was chosen because (a) previous studies showed that it supported maximum internodal elongation (Adams et al., 1973; Montague et al., 1973), (b) a carbohydrate source has been shown to support the gravitropic response of the leaf-sheath pulvinus (Bridges and Wilkins, 1973), and (c) readily available carbohydrate more closely mimics the usual condition within the plant, where adequate levels of substrate presumably exist. In some experiments, noted in the figure legends, 10 mM Mes (NaOH), pH 5.5, was also included in the incubation medium, although no effect on internodal elongation was found. Segments were allowed to grow at 30°C in the dark enclosed in plastic containers at 100% RH, with provision for some air exchange. The lengths of the internodes and pulvini were measured to the nearest 0.5 mm with a ruler under a dim green safelight using a magnifying lens. In some cases, the outlines of the pulvini were traced on paper and then measured.

In experiments designed to study the participation of cell wall synthesis in gravitropism, whole segments were placed in 0.5-mL Eppendorf centrifuge tubes. The node of each segment was in contact with a small piece of U.S. Pharmacopeia-grade cotton packed in the bottom of the tube and saturated with 0.2 mL of 0.1 M [^{14}C]Glc, which in some cases contained IAA or GA_3 as well. The tubes were placed upright in a test tube rack (control vertical segments) or the rack was tilted 90° to provide the gravistimulus to the pulvini. Segments were incubated at 30°C in the dark as described above.

The size (fresh weight) of individual segments was highly correlated with total uptake of label (M.J. Montague, unpublished data). For this reason, shoots were carefully selected to enhance uniformity of size and developmental stage for each experiment within the limits of the availability of plant material. Although the initial mean length of the internodal tissue was quite similar in different batches of segments (ranging from 6–8 mm), the average length of the pulvini ranged from about 1.5 to 3.5 mm. The differences in pulvinar length seemed to be positively correlated with the width of the shoot, which varied even for shoots grown in the same pot. In part because of this batch-to-batch variability, all key findings were repeated at least three times in alternate experimental contexts and with unrelated batches of segments. Statistical analyses were performed as appropriate to provide an indication of internal variability, and details are given in the figure legends.

Cell Wall Preparation and Extraction

In general, cell wall material was prepared using the method of Baker and Ray (1965). Oat pulvini or internodes were frozen immediately on dry ice and were then extracted with 80% (v/v) ethanol for at least 24 h at room temperature, the ethanol was collected, and the tissue was extracted once or twice more with fresh 80% (v/v) ethanol for at least an additional 24 h. The ethanol extracts were combined and counted. The tissue was crushed either between two glass plates or with the end of a glass rod and placed in a pepsin (porcine stomach mucosa) solution (1040 units/mL in 30 mM potassium phosphate buffer, pH 2) overnight, after which the solution was removed and counted.

The resultant cell wall material was routinely hydrolyzed in 1 mL of 2 M TFA at 121°C for 1 to 1.5 h. This procedure separates the matrix (TFA soluble) from cellulose (TFA insoluble) components (Brummell and Hall, 1983), which are so designated herein. One caution in interpretation, however, pointed out by Mankarios et al.

(1979), is the likelihood that the less crystalline cellulose of primary cell wall may be hydrolyzed given sufficient time. The TFA-soluble material was counted directly and the insoluble cellulose residue was dissolved in 72% (v/v) H_2SO_4 overnight before counting. To obtain greater resolution of cell wall components in some experiments, the pepsin-treated wall material was extracted overnight with 1 mL of porcine pancreatic α-amylase (58 units/mL in 10 mM Tes [NaOH] buffer, pH 7.0) and then with 1 mL of hot water (121°C for 15 min) prior to the TFA extraction. Routine extraction of starch was judged unnecessary because of initial results obtained with α-amylase digestion, as well as the results of Gibeaut et al. (1990), who found that starch constituted only about 1% of the cell wall material isolated from oat pulvini. Radioactivity was determined using Instagel XF scintillation fluid (Packard, Meriden, CT). Uptake was considered as the combined solubilized and cell wall fractions. Any $^{14}CO_2$ produced was not measured, although previous results (Montague and Ikuma, 1975) showed that it represented about 15% of the radioactivity recovered from isolated internodes, with little difference between untreated and GA_3-treated segments.

Chemicals

FC, IAA, GA_3, pepsin, and α-amylase were obtained from Sigma. FC and IAA were dissolved in methanol before addition to the growth medium. The methanol concentration never exceeded 1% and an identical concentration was supplied to control tissue. D-[U-^{14}C]Glc (9.5 GBq/mmol) was purchased from New England Nuclear. All other chemicals were of reagent grade.

RESULTS

Anatomy of the *Avena* Stem Segment

A schematic representation of the *Avena* stem segment system is shown in Figure 1 (for a monograph on oat anatomy and development, see Kaufman and Brock, 1992; for photographs of this system, see Adams et al., 1973; Kaufman and Dayanandan, 1983). Note the central internode portion, which responds to GA_3 by elongation produced entirely by cell expansion without concomitant cell division (Kaufman et al., 1969). The internode is attached at its base to the node, which contains anastomosing vascular bundles (Montague et al., 1973). Also attached to the node is the leaf sheath, with its graviresponsive, auxin-sensitive pulvinus located at the base.

Kinetics of Gravistimulated Pulvinar Growth

Other workers (Kaufman and Dayanandan, 1985; Brock and Kaufman, 1988b), using 80-mm *Avena* segments to study the response of the pulvinus to gravity, have reported the initiation of bending by 0.5 to 1 h. Although preliminary experiments also showed significant gravistimulated bending of the 10-mm segments used here, this response was difficult to measure accurately, especially at early times, because the segments were so short. Therefore, elongation of the lower half of gravistimulated pulvini was chosen as an alternative method to determine the kinetics of the pulvinar growth response. Segments were unilaterally marked with ink on the leaf sheath to distinguish one side from the other. Elongation of pulvini on the marked side (the lower side in gravistimulated segments) was determined during a 48-h time course (Fig. 2). The results show that pulvini from vertical segments did not elongate, and visual observation revealed that the vertical segments did not bend. The lower halves of gravistimulated pulvini did elongate, however, becoming significantly different from the control between 3 and 6 h after gravistimulus, with growth continuing at a reduced rate for at least 48 h.

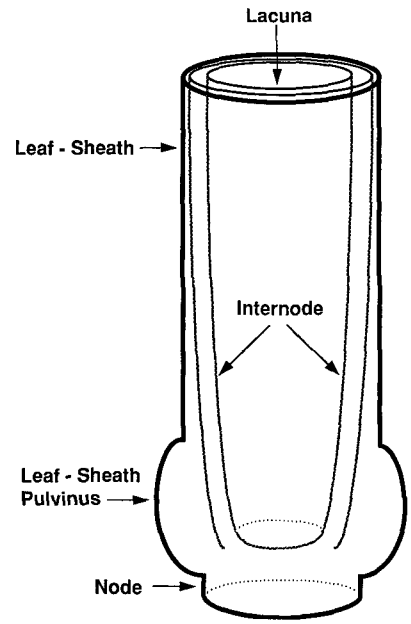

Figure 1. Schematic diagram of whole *Avena* stem segment. The segment is shown in the vertical orientation. Vascular elements from the leaf sheath and internode anastomose in the node.

Early Kinetics of Cell Wall Synthesis in Gravistimulated Pulvini

Several preliminary experiments showed that gravistimulus also promoted the incorporation of [^{14}C]Glc into cell wall material from pulvini of 10-mm *Avena* stem segments. Figure 3 shows a representative example of the early time course of the response. Note that at 1 and 3 h, uptake and incorporation into cell wall were statistically indistinguishable between gravistimulated and vertical pulvini. At 6 h, however, gravistimulus resulted in a 2.2-fold promotion of cell wall incorporation compared with the vertical control, whereas the promotion of uptake was less pronounced (1.7-fold). The percentage of uptake found in cell wall material was higher in gravistimulated pulvini compared with the control (19% compared with 15%), whereas the percentages of wall label found in the cellulose fractions were similar for the two treatments (about 35%). This result shows that gravistimulus promoted the incorporation of labeled Glc into both fractions of the cell wall, with the effect initiated between 3 and 6 h. These kinetics

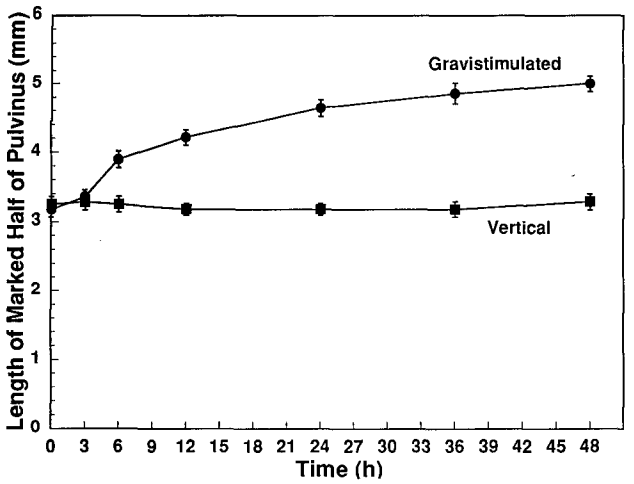

Figure 2. Effect of gravistimulus on growth in pulvini from *Avena* stem segments. Samples of 14 *Avena* stem segments were marked unilaterally with ink on the leaf sheath and then were incubated with 0.1 M Glc in the absence or presence of gravistimulus (marked side down). At the indicated times, the lengths of pulvini on the marked sides were measured. Mean lengths are presented with SE.

are remarkably similar to the kinetics of growth shown in Figure 2.

Responses of Internode and Pulvinus to Exogenous IAA

Although the exact role of auxin in the gravitropic response of grass pulvini is unknown, there is substantial evidence that at least some of the effects of gravistimulus can be mimicked by IAA (Bridges and Wilkins, 1973; Brock and Kaufman, 1988a). To investigate the comparison further, the responses of both the internode and pulvinus of vertical *Avena* stem segments to the application of a range of concentrations of exogenous IAA were determined. In this experiment (Fig. 4), vertical stem segments were supplied with IAA (0–1.0 mM) in the presence of 0.1 M [^{14}C]Glc for 10.5 h. Figure 4A shows total average length and cell wall radioactivity for the internode portion. IAA had no statistically significant effect on the average length of the internodal tissue; nor was there any consistent effect on the fresh weight per segment. Although the hormone did not alter the amount of radioactivity incorporated into either the matrix or cellulose components of the cell wall, there was a dose-dependent reduction in uptake, with internodes given 1.0 mM IAA displaying only 60.2% as much uptake of label as untreated internodes. Thus, the percentage of label incorporated into the cell wall actually increased.

The responses of the pulvini to IAA are presented in Figure 4B. A consistent dose-dependent increase in fresh weight per pulvinus was found with increasing concentration of IAA. At 1.0 mM IAA, the mean fresh weight was 1.4-fold higher than without hormone. This increase in fresh weight was reflected in obvious lengthening of the pulvini (also see Fig. 6). In addition, there was a dose-dependent increase in incorporation of radioactivity into both the matrix and cellulose components of the cell wall. Total cell wall incorporation was 4.8-fold higher with 1.0 mM IAA than without hormone, whereas uptake was only 2.1-fold higher.

These results portray a clear difference in responsiveness of the internodal and pulvinar tissues to auxin. The internode showed no response to IAA, except for a decrease in uptake of [^{14}C]Glc, which may have occurred simply because of a greater relative movement of radioactivity into the pulvinus. Note that the absolute level of incorporation of labeled Glc into internodal cell wall material was maintained, and therefore, the amount of radioactivity in the cell wall as a percentage of uptake increased. In contrast, the pulvinus responded to increasing concentrations of IAA with increased fresh weight, uptake of label, and incorporation into both the matrix and cellulose components of the cell wall.

Early Kinetics of IAA- and FC-Promoted Cell Wall Synthesis in the Pulvinus

FC has been used effectively as a nonphysiological tool to probe hormone-dependent processes in a variety of plant systems and, in particular, to mimic some of the effects of auxin (Marrè, 1979; Brummell and Hall, 1983). To determine whether FC could mimic the effects of IAA on oat pulvini, the early time course of auxin-promoted cell wall synthesis in vertical *Avena* stem segment pulvini was determined and compared with the effects of FC (Fig. 5). The rate of uptake of radioactivity by pulvini from segments treated only with [^{14}C]Glc decreased during the 6-h time course (Fig. 5A). Both 1.0 mM IAA and 10 μM FC stimulated uptake at all times compared with this Glc control. The percentage of label incorporated into cell wall material (Fig. 5B) for both IAA and FC treatments was greater than the control, however, indicating that absorbed label was preferentially incorporated into cell wall. The kinetics of incorporation of radioactivity into the cell wall were quite different in pulvini treated with IAA and FC compared with the control (Fig. 5C). By 3 h the rate of incorporation of radioactivity into cell wall in the Glc control had declined, whereas the rates for IAA and FC were considerably greater than the control. By 6 h IAA-treated pulvini and FC-treated pulvini had incorporated 3.3- and 4.4-fold, respectively, as much label as the Glc control. Although treatment with IAA slightly reduced the relative amount of incorporation into cellulose compared with matrix polysaccharide (Fig. 5D), this effect was much greater in the FC-treated segments.

These results show that both IAA and FC promoted the incorporation of radioactivity into cell wall material from oat pulvini. The effect could be demonstrated 1 h after the start of incubation and became progressively more pronounced during the 6-h time course. Although IAA and FC also promoted uptake of label, this was not as great as their effect on the cell wall. Apparently, new cell wall synthesized with FC treatment contained proportionately more matrix polysaccharide than the control or at least the radioactivity was more easily extractable with hot 2 M TFA. These results (Figs. 4 and 5) show that IAA is able to mimic at least some of the effects of gravistimulus on cell wall

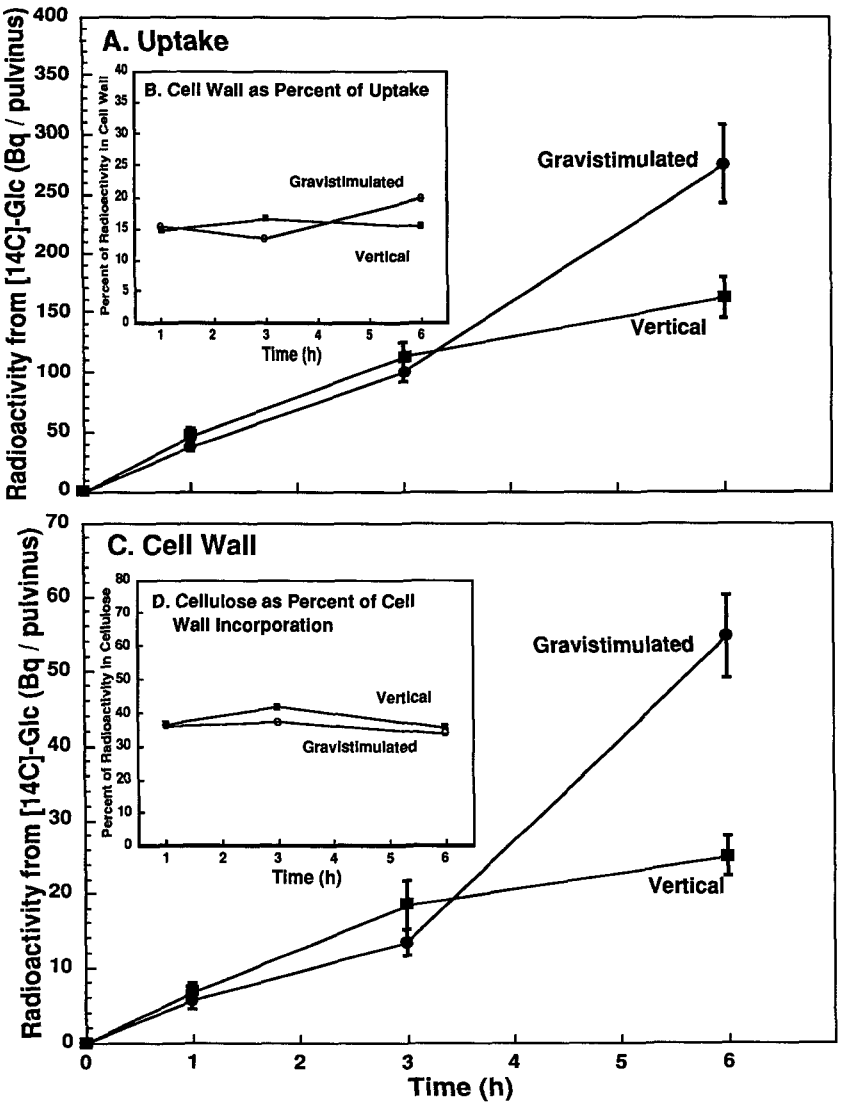

Figure 3. Effect of gravistimulus on cell wall synthesis in pulvini from *Avena* stem segments. Samples of 10 *Avena* stem segments were incubated with 0.1 M [^{14}C]Glc (1.4 kBq/μmol) with or without gravistimulus for the indicated times. Pulvini were collected and extracted in five lots of two segments for each point. A, Uptake of label into all fractions. Mean values are presented with SE (computed as the SE of five individual groups of two pulvini each). B, Percentage of label found in cell wall (matrix and cellulose components combined). C, Radioactivity found in combined matrix and cellulose components of the cell wall. Mean values presented with SE, as above. D, Percentage of radioactivity in wall present in the cellulose component.

synthesis in oat pulvini. Moreover, the initiation of enhanced cell wall synthesis occurs earlier with IAA and FC treatment than with gravistimulation in these 10-mm segments.

Comparison of the Responses of Internode and Pulvinus to GA_3, IAA, and FC

FC has been used less often to mimic the growth-promoting effects of GA and there appear to be greater differences between the action of FC and GA compared with auxin (Stuart and Jones, 1978). Nonetheless, an examination of the potential of FC to stimulate cell wall synthesis in a tissue so specifically responsive to GA was an attractive option. Internodal growth and radioactivity from [^{14}C]Glc as found in five different fractions (80% ethyl alcohol, pepsin, α-amylase, hot 2 M TFA, and 72% sulfuric acid) are reported in Figure 6, A and B, for an experiment in which IAA, FC, and GA_3 were supplied at 1.0 mM, 10 μM, and 0.5 mM, respectively, for 10.5 h. As in Figure 4A, IAA supplied alone at 1.0 mM caused a decrease in the internodal uptake of label, with little effect either on elongation or on incorporation into cell wall. FC decreased uptake even more but promoted elongation and incorporation into the matrix (TFA soluble) component of the cell wall. As shown in Figure 5D for the pulvinus, FC caused a decrease in the percentage of label found in the cellulose component of the internodal cell wall (24.7% compared with 39.5% in the Glc control). As expected from previous work (Montague and Ikuma, 1975), GA_3 strongly promoted elongation, uptake, and incorporation into both the matrix and cellulose components of the wall.

The results for the pulvinar tissue are presented in Figure 6, C and D. The pattern of promotion and inhibition here was quite different from the internode. In the pulvinus, IAA promoted elongation, uptake, and incorporation into the matrix (3.8-fold) and cellulose (4-fold) components of the wall. This is consistent with the results presented in Figures 4 and 5. The effect of FC on elongation and incorporation into the cellulose component of the cell wall was

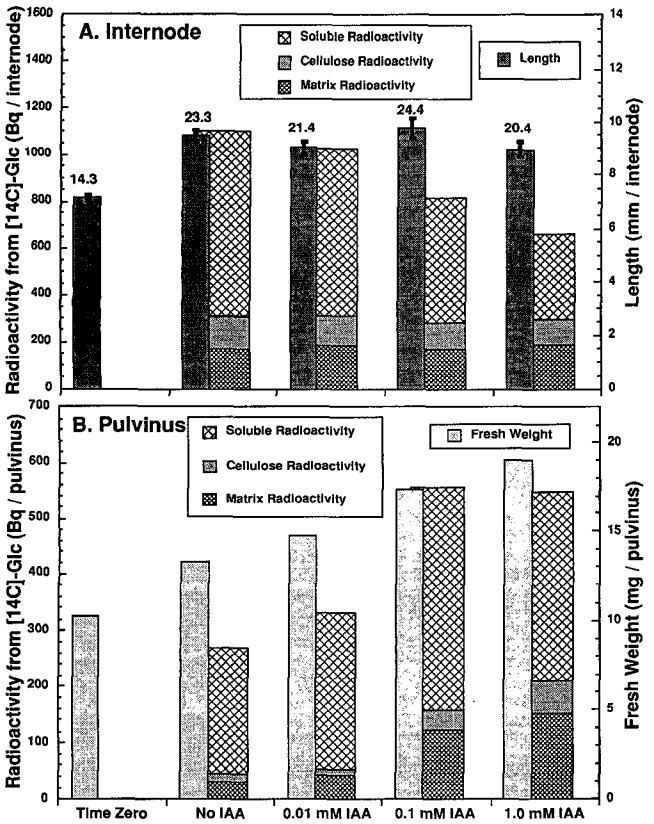

Figure 4. *Effect of IAA on the internodes and pulvini from whole Avena stem segments. Samples of 11 segments were incubated with 0.1 M [^{14}C]Glc (1.1 kBq/μmol) in the presence or absence of the indicated concentrations of IAA for 10.5 h. A, Lengths, fresh weights, and soluble and cell wall radioactivity of the dissected internodal portions. Mean lengths are shown with SE. Figures above bars show fresh weight (mg) per internode. B, Fresh weights and soluble and total cell wall radioactivity of the dissected pulvini.*

not significantly different from IAA, but it caused substantially greater incorporation into the matrix component (8.6-fold greater than the Glc control and 2.3-fold greater than IAA). Thus, as in Figure 5D, the percentage of label found in cellulose decreased (15.4% compared with 37.5% in the IAA-treated pulvinus). GA_3 caused no statistically significant effect on elongation, uptake, or cell wall incorporation.

Taken together these results demonstrate clear and apparently specific differences between the effects of IAA, FC, and GA_3 in these two growth-capable portions of the *Avena* stem segment. IAA stimulated growth (fresh weight increase and elongation) and cell wall synthesis in the pulvinus but decreased uptake by the internode with little effect on growth or wall incorporation. FC stimulated elongation in both the internode and pulvinus. Although it inhibited uptake by the internode, it stimulated uptake by the pulvinus. In addition, it promoted incorporation into the matrix component of the internodal wall and into both components of the pulvinar wall. For both tissues, FC caused a decrease in the relative percentage of label found in the cellulose component of the wall. Judged by the magnitude of the promotion of uptake and wall synthesis, the pulvinus is considerably more responsive to FC than is the internode. As expected, GA_3 strongly promoted elongation, uptake, and wall incorporation in the internode, with little effect on the pulvinus other than a slight (not statistically significant) inhibition of uptake. The promotion of wall synthesis by IAA and the absence of any promotion by GA_3 in vertical pulvini were observed repeatedly and consistently from experiment to experiment. Attempts to show enhancement by GA_3 of the IAA-promoted synthesis of cell wall material in the pulvinus were unsuccessful (data not shown). Attempts to demonstrate IAA-promoted cell wall incorporation in pulvini isolated from the stem segment were also unsuccessful (data not shown).

Effect of Gravistimulus on the Response of the Pulvinus to IAA

From the data reported above, it is clear that IAA mimics two effects of gravistimulus in pulvini from oat stem seg-

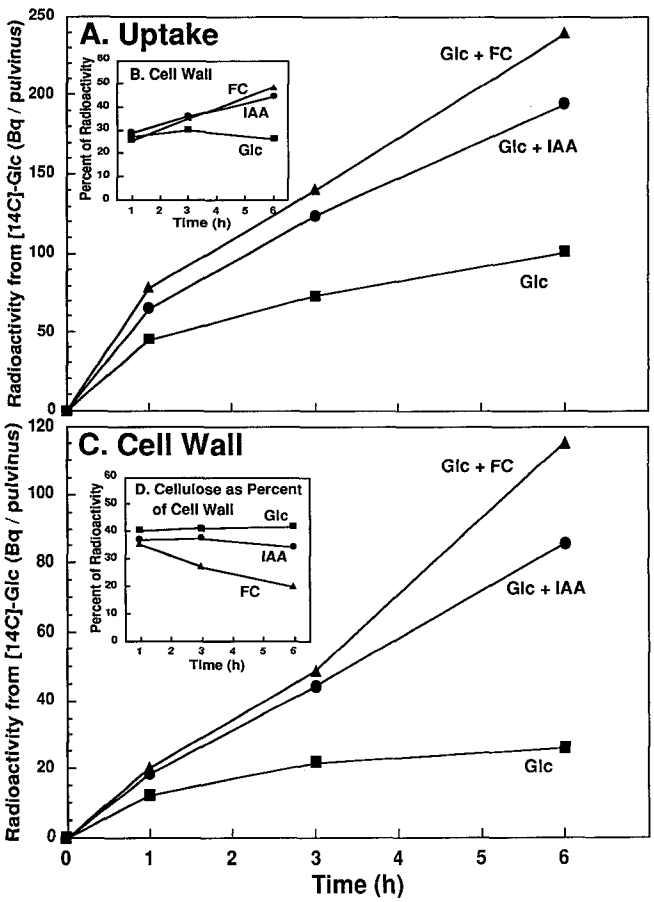

Figure 5. Effects of IAA and FC on cell wall synthesis in pulvini from whole *Avena* stem segments. Samples of five to seven stem segments were incubated with 0.1 M [^{14}C]Glc (0.8 kBq/μmol) for the indicated times in the presence or absence of 1.0 mM IAA or 10 μM FC. A, Uptake of radioactivity per pulvinus. B, Percentage of label incorporated into cell wall. C, Incorporation of radioactivity into combined matrix and cellulose components of the cell wall. D, Percentage of wall label found in the cellulose fraction.

ments, namely increased growth (Figs. 4B and 6D) and early promotion of cell wall synthesis (Fig. 5C). To determine whether gravistimulus affects the response or sensitivity of the pulvini to auxin, stem segments fed 0.1 M [^{14}C]Glc were gravistimulated for 11.5 h in the presence or absence of 10 μM IAA, a concentration at the low limit of effectiveness in vertical segments (Fig. 4B). Figure 7 shows results of incorporation of radioactivity into six different solvent fractions (80% ethyl alcohol, pepsin, α-amylase, hot water, hot 2 M TFA, and 72% H_2SO_4). Gravistimulus by itself stimulated incorporation into all six fractions, with disproportionately more stimulation into the cell wall fractions (combined 2 M TFA and 72% H_2SO_4-soluble), in which the ratio of cell wall incorporation in gravistimulated versus vertical segments was about 3-fold. IAA applied to vertical segments slightly stimulated incorporation into the combined wall fractions (by 16.4%), but neither the matrix nor cellulose components were statistically distinguishable from the control vertical pulvini. When applied to gravistimulated segments, IAA increased total cell wall incorporation over the non-hormone-treated, gravistimulated control (by 19.4%), but the difference was small and again the individual means for matrix and cellulose components were not significantly different. The results show that gravistimulus does not increase the responsiveness of oat pulvini to IAA, as measured using cell wall incorporation, which is in agreement with the findings of Brock and Kaufman (1988a), who studied the same interaction but measured the bending response.

Effect of Gravistimulus on the Response of Internode and Pulvinus to GA_3

Brock and Kaufman (1988a) reported a relatively small stimulation of gravistimulated bending by 30 μM GA_3. To examine the interaction between GA_3 and gravistimulus further, internodal and pulvinar tissues from *Avena* stem segments, incubated with [^{14}C]Glc for 10.5 h with or without gravistimulus and with or without 30 μM GA_3, were collected and fractionated simultaneously. Figure 8A documents the incorporation of radioactivity into five different solvent fractions (80% ethyl alcohol, pepsin, α-amylase, hot 2 M TFA, and 72% H_2SO_4) from the internode. As expected, GA_3 significantly increased the amount of radioactivity found in all fractions regardless of gravistimulation. By itself, gravistimulus caused a small reduction in wall incorporation (both matrix and cellulose fractions) and this reduction was paralleled in the presence of hormone. The differences for wall incorporation were not statistically significant, however. The same trends were also paralleled in the effect of gravistimulus on internodal elongation (Fig. 8B). Thus, gravistimulus had essentially no effect on the internodal portion of these segments.

The interaction between gravistimulus and GA_3 in the pulvinar tissue from the same segments is shown in Figure 8C. The pattern of label present in the five fractions is quite different from Figure 8A. First, in vertical segments, GA_3 had no effect on either uptake or wall incorporation, consistent with the findings of Brock and Kaufman (1988a) and Figure 6. Second, gravistimulus promoted incorporation

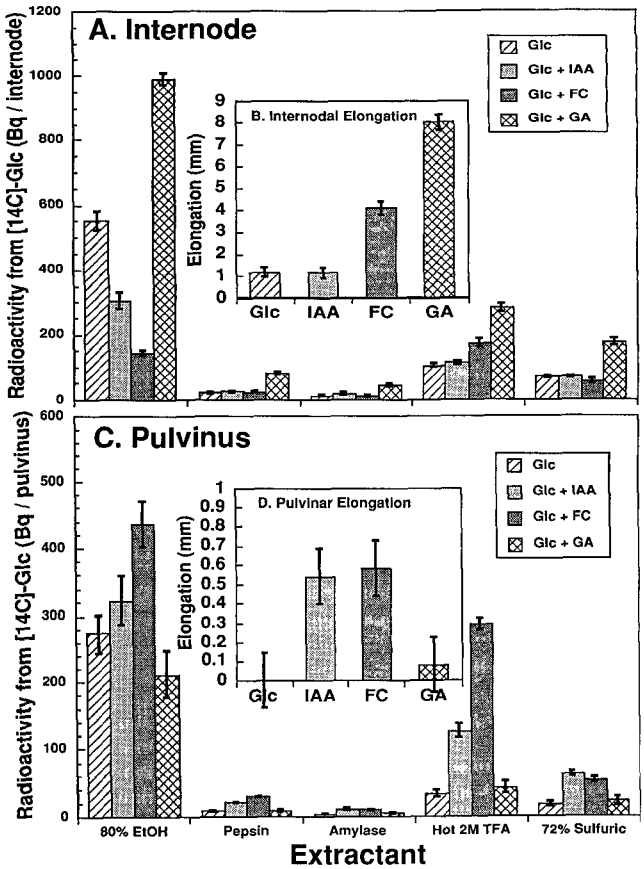

Figure 6. Effects of IAA, FC, and GA_3 on growth and cell wall synthesis in internodes and pulvini from whole *Avena* stem segments. Samples of 12 segments were incubated with 0.1 M [^{14}C]Glc (1.1 kBq/μmol) and 10 mM Mes, pH 5.5, in the presence or absence of 1.0 mM IAA, 10 μM FC, or 0.5 mM GA_3 for 10.5 h. Lengths of internodes (7.6 ± 0.13 mm) and pulvini (1.7 ± 0.10 mm) were determined on time-zero samples so that net growth (elongation) could be determined. Internodal and pulvinar tissues were collected and extracted in four lots of three segments for each treatment. A, Radioactivity present in the indicated fractions from the internodal tissue. Means are presented with SE (computed as the SE of four individual groups of three internodes each). B, Elongation of the internodes. Means are presented with SE, computed from the pooled variance of both time-zero and treated internodes. C, Radioactivity present in the indicated fractions from the pulvinar tissue. Means are presented with SE as in A. D, Elongation of the pulvini. Means are presented with SE as in B.

into all five fractions, although disproportionately into the wall fractions (to an even greater extent than shown in Fig. 7). Third, treatment with GA_3 reduced uptake and incorporation into both cell wall fractions when the segments were gravistimulated. Thus, treatment with GA_3 reduced the movement of label into the pulvinar tissue. It seems likely that the stimulation of internodal growth created a large demand for Glc that "pulled" label from the pulvinus, making it unavailable for incorporation into the pulvinar cell wall. Nonetheless, even in the presence of GA_3-induced internodal growth, it is worth noting that gravistimulus still significantly enhanced pulvinar cell wall in-

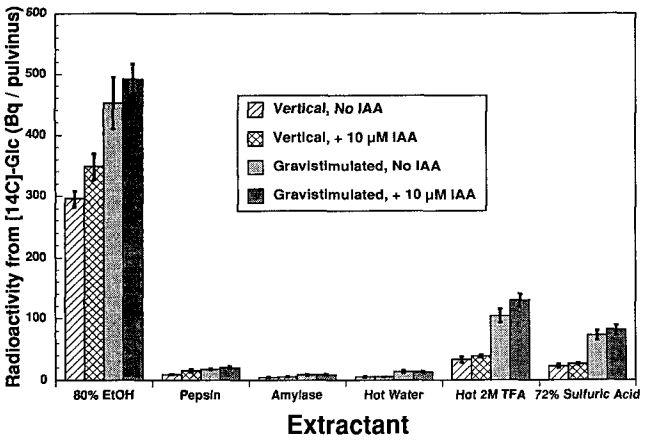

Figure 7. Effect of gravistimulus on the response of *Avena* pulvini to IAA. Samples of 12 segments were incubated with 0.1 M [^{14}C]Glc (1.7 kBq/µmol) in the presence or absence of gravistimulus and/or 10 µM IAA. Pulvini were collected and extracted in lots of three after 11.5 h. Results are presented with SE, computed as the SE of four individual groups of three pulvini each. EtOH, Ethanol.

corporation compared with the hormone-treated, vertical control and the reduction in wall incorporation (both components combined) was less (40.1%) than the reduction in uptake (49.1%).

Responses of Whole Stem Segments and Isolated Internodes to GA_3 and FC

The internodal tissue dissected from *Avena* stem segments is capable of elongating in response to treatment with GA_3, although neither the peak rate nor maximum elongation is as great as when the internode remains attached to the node and leaf sheath (Montague et al., 1973). Results of an experiment to compare the responses of internodal tissue attached as usual to the node and leaf sheath (whole segments) with internodes dissected away (isolated internodes) are shown in Table I. GA_3 and FC stimulated growth in both cases, measured either as elongation or as increased fresh weight. The FC-promoted swelling observed with internodal tissue from whole segments was more pronounced in the isolated internodes (reflected as fresh weight per unit length). These results show that the presence of node and leaf sheath is not required for stimulation of growth in internodal tissue from *Avena* stem segments by FC, although the orientation of growth (elongation compared with lateral expansion) is different in the two cases.

Kinetics of FC- and GA_3-Promoted Internodal Elongation

Because treatment with FC clearly promoted elongation and cell wall synthesis in GA-sensitive oat internodal tissue at a relatively early time, it seemed useful for comparative purposes to examine the long-term kinetics of the response. The results presented in Figure 9 show a GA_3-induced growth response in whole segments similar to that reported previously (Montague, 1993). FC also promoted elongation early in the time course. In fact, the elongation obtained with FC or GA_3 was comparable at 6 h. After that time, FC produced substantially less elongation than GA_3, slowed by 12 h, and was complete at about 23 h, whereas GA_3-induced growth continued for at least 80 h. When FC and GA_3 were supplied together (an experiment rarely reported in the literature for any hormone), FC was found to inhibit the response to GA_3, resulting in growth nearly identical with that obtained with FC alone. Apparently, the toxic effects of FC prevented the normal continuation of the GA_3 response in this system.

Early Kinetics of FC- and GA_3-Promoted Internodal Elongation and Cell Wall Synthesis

Results reported previously (Montague and Ikuma, 1975) using isolated internodes that had been pretreated for 12 h with 0.1 M Glc showed that GA_3 promoted both elongation and cell wall synthesis starting about 1 h after hormone

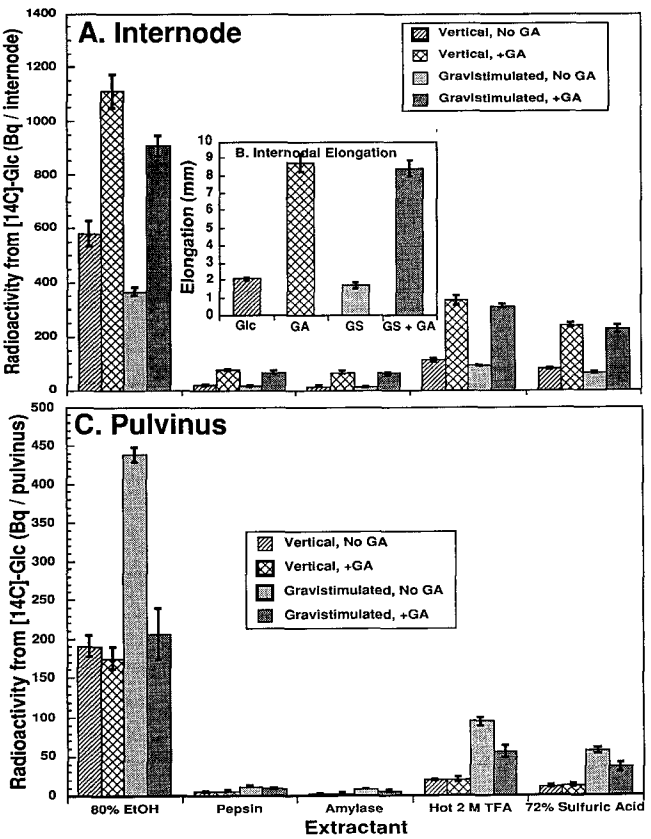

Figure 8. Effect of gravistimulus on the response of *Avena* internodes and pulvini to GA_3. Samples of 12 segments were incubated with 0.1 M [^{14}C]Glc (1.3 kBq/µmol) in the presence or absence of gravistimulus (GS) and/or 30 µM GA_3 for 10.5 h. Internodal and pulvinar tissues were collected and extracted in four lots of three segments for each treatment. A, Radioactivity present in the indicated fractions from the internodal tissue. Means are presented with SE (computed as the SE of four individual groups of three internodes each). B, Elongation of the internodes. Means are presented with SE. C, Radioactivity present in the indicated fractions from the pulvinar tissue. Means are presented with SE as in A.

application. From the results in Figure 10 using whole stem segments, treatment with GA_3 or FC caused increased elongation in internodal tissue that remained attached to the node and leaf sheath. The effects of the two stimuli were observable by 2 h (Fig. 10A). Both compounds promoted the uptake of [^{14}C]Glc (Fig. 10B) and incorporation into cell wall (Fig. 10C) as well. The effect of GA_3 on wall synthesis was slightly more pronounced than that of FC. Also, as with isolated internodes (Montague and Ikuma, 1975), the effect of GA_3 was observed by 2 h and, therefore, was initiated between 1 and 2 h after the start of incubation. Both stimuli promoted cell wall incorporation proportionately more than they promoted the uptake of label (Fig. 10D), indicating preferential movement of radioactivity into the wall. As above (Fig. 6A), FC stimulated relatively more incorporation into the matrix than into the cellulose component of the cell wall compared with GA_3 or the Glc control.

In this experiment, the percentages of total wall incorporation identified as cellulose were 30.3 and 24.2% for GA_3 and FC, respectively, at 3 h. In another similar experiment, the values were 39.7 and 24.0%, respectively, at 6 h. It is worth noting that the kinetics of growth induction and enhanced cell wall synthesis were quite similar for GA_3 and FC. The average times of induction of elongation by GA_3 in *Avena* stem sections reported in the literature range widely, from 78 min (Adams and Ross, 1983) to 3.5 h (Kaufman and Dayanandan, 1983). Various pretreatment conditions, such as with IAA (Adams and Ross, 1983), and the physiological state of the tissue, especially turgor status, can alter the time of induction by several hours (M.J. Montague, unpublished data).

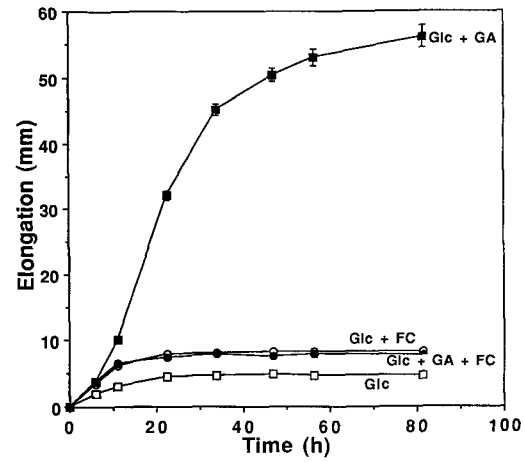

Figure 9. Effects of GA_3 and FC on long-term elongation of whole *Avena* stem segments. Samples of 12 stem segments were incubated with 0.1 M Glc in the presence or absence of 0.5 mM GA_3 or 10 μM FC for the indicated times, at which elongation of the internode was measured. Mean lengths are given with SE.

DISCUSSION

Kaufman and co-workers (for review, see Kaufman and Dayanandan, 1985; Kaufman et al., 1987; Brock and Kaufman, 1990) have extensively characterized the physiology and biochemistry of the response of the leaf-sheath pulvini of oat and barley to gravistimulation. This agronomically important bending response is initiated within 0.5 to 1 h following appropriate gravistimulus of the pulvinus (Kaufman and Dayanandan, 1985; Brock and Kaufman, 1988b, 1988c). Strong evidence exists that the initial graviperception occurs through the movement of starch statoliths (amyloplasts) located in the statenchyma cells found near each vascular bundle in the pulvinus (Brock and Kaufman, 1988c; Song et al., 1988). The subsequent differential growth response apparently does not involve cell division but rather occurs solely through a gradient of cell elongation, with the greatest response found in the collenchyma located in the downward-facing half of the pulvinus (Dayanandan et al., 1976). Unilaterally applied auxin causes differential lengthening of the pulvinus, thereby bending the shoot (Dayanandan et al., 1976; Brock and Kaufman, 1988a). Lateral movement of IAA does not appear to be required for the gravitropic response, however (Bridges and Wilkins, 1973; Brock et al., 1991). These observations and measurement of free auxin levels have led to the hypothesis that gravistimulation may trigger a release of

Table I. *Effects of GA_3 and FC on growth in whole (intact) and isolated internode segments from Avena*

Samples of 11 *Avena* stem segments (either whole or isolated internodes) were incubated for 12 h with 0.1 M Glc in the presence or absence of 0.5 mM GA_3 or 10 μM FC. Total lengths and fresh weights were determined for the internode portions of the segments.

Treatment	Growth of Internode Portion				
	Total mean length	Mean length increase	Mean fresh wt	Net fresh wt increase	Fresh wt/unit length
	mm ± SE	*mm*	*mg segment^{-1}*	*mg segment^{-1} 12 h^{-1}*	*mg mm^{-1}*
Whole segment					
Time zero	7.6 ± 0.15		17.1		2.3
Glc	9.8 ± 0.18	2.2	23.5	6.4	2.4
Glc + GA	22.2 ± 0.46	14.6	41.4	24.3	1.9
Glc + FC	12.2 ± 0.26	4.6	31.9	14.8	2.6
Isolated internode					
Time zero	10.0 ± 0.00		23.5		2.4
Glc	12.3 ± 0.14	2.3	30.9	7.4	2.5
Glc + GA	20.1 ± 0.34	10.1	47.0	23.5	2.3
Glc + FC	12.7 ± 0.19	2.7	36.9	13.4	2.9

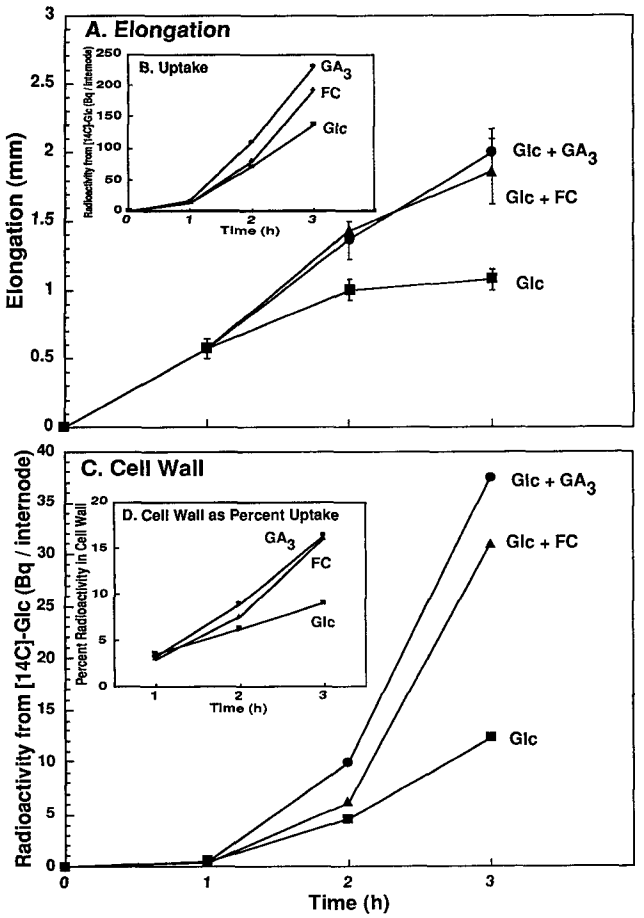

Figure 10. Effects of GA_3 and FC on growth and cell wall synthesis in internodes from whole *Avena* stem segments. Samples of 7 stem segments were pretreated on water at 4°C for 22 h before incubation with 0.1 M [^{14}C]Glc (1.1 kBq/μmol) for the indicated times in the presence or absence of 0.5 mM GA_3 or 10 μM FC. A, Elongation of the internode portion. Mean lengths are given with SE. B, Uptake of radioactivity into combined soluble and cell wall fractions. C, Incorporation of radioactivity into combined matrix and cellulose components of the cell wall. D, Radioactivity in total cell wall as a percentage of uptake.

IAA from its conjugates, which in turn causes enhanced cell elongation at the site (Kaufman and Dayanandan, 1985; Brock and Kaufman, 1988a).

Studies of the involvement of cell wall synthesis in the gravitropic response of oat pulvini have produced contradictory results. Using a wall isolation protocol different from that used here, Gibeaut et al. (1990) reported significant increases in cell wall dry weight in the upward-facing and especially in the downward-facing halves of gravistimulated oat pulvini compared with the vertically held control. Although no data were presented, these authors reported that all wall fractions, including cellulose, increased proportionately upon gravistimulation. The only exception was a somewhat greater increase in β-1,3-glucan in the downward-facing halves, in which higher levels of glucan synthase activity (product uncharacterized) were found as well. In contrast, Lu et al. (1992) used ultrastructural analysis to show a decrease in the density of material within the cell walls from the lower halves of oat pulvini after 24 h of gravistimulation. Moreover, even using [^{14}C]Suc pulse labeling (apparently chased with continuously supplied 0.1 M Suc), they were unable to demonstrate enhanced cell wall synthesis in the bottom (downward facing) halves compared with the top halves of gravistimulated pulvini. Based on these and additional enzyme and inhibitor data, they concluded that the graviresponse was not driven by new cell wall synthesis.

The present results are in accord with those of Gibeaut et al. (1990). Gravistimulus clearly led to a significant increase in the incorporation of [^{14}C]Glc into the cell wall (Figs. 3C, 7, and 8C). The response was documented as early as 6 h after initiation of gravistimulation, with kinetics similar to the kinetics of elongation of the lower half of the pulvinus (cf. Figs. 2 and 3). Gravistimulus promoted the synthesis of both the matrix and cellulose components of the wall. This finding is consistent with those of Gibeaut et al. (1990), who also reported (relying on differential solubility and chemical identification but showing no data) that all components of the wall increased proportionately in response to gravistimulation. They also found that about 32% of the pulvinar cell wall material was cellulose, a value quite similar to that reported here (about 35%) derived using an alternative extraction method and measuring incorporation of label rather than absolute amount. In the present work, enhanced cell wall synthesis and elongation of the lower half of gravistimulated pulvini were both initiated between 3 and 6 h. This is significantly later than the 0.5 to 1 h time of initiation of bending found with 80-mm segments used by others (Kaufman and Dayanandan, 1985; Brock and Kaufman, 1988b). Unfortunately, accurate measurement of the initiation of bending was not possible with the relatively short 10-mm segments used here; therefore, the results are not strictly comparable. Of course, the difference in time of initiation could be due at least in part to the greater amount of tissue present in the 80-mm segments. Note that the lag time is certainly influenced by the developmental stage of the plant (Brock and Kaufman, 1988b). In addition, no conclusions should be drawn from these data about an absolute requirement for enhanced cell wall synthesis in the pulvinar graviresponse.

Brock and Kaufman (1988a) showed convincingly that the gravitropic bending response in oat pulvini could be mimicked by IAA, albeit at fairly high concentrations (100 μM), applied unilaterally to the pulvinus. Results from the present study show that IAA, at similar concentrations but delivered through the normal vasculature in the node of the whole segment, promoted cell wall synthesis as well (Figs. 4B, 5C, and 6C). Whereas gravistimulus leads to a decided gradient of elongation within the pulvinus (Dayanandan et al., 1976), IAA supplied through the node caused a dose-dependent, uniform lengthening of the pulvinus, accompanied by increased fresh weight, enhanced uptake of label, and increased incorporation into both the cellulose and matrix components of the cell wall (Fig. 6C). The auxin effect on cell wall synthesis could be observed by 1 h after application of the hormone (Fig. 5C), which is

Table II. *Qualitative summary of the effects of gravistimulus, IAA, GA_3, and FC on growth and cell wall synthesis in internodes and pulvini from Avena stem segments*

0, No effect; +, promotion.

Tissue	Treatment			
	Gravistimulus	IAA	GA_3	FC
Internode	0[a]	0[a]	+	+
Pulvinus	+	+	0	+

[a] Had no effect on internodal growth but led to reduced uptake of label.

comparable to the kinetics observed in other auxin-sensitive elongating systems such as pea stem sections (Abdul-Baki and Ray, 1971) and oat coleoptiles (Baker and Ray, 1965). As further validation, it is important to note that the promotion by auxin of wall incorporation was greater proportionally than its promotion of uptake (Figs. 5B and 6C).

The interactions between hormones and gravistimulus are revealing from a mechanistic standpoint. Bridges and Wilkins (1973) concluded that exogenously supplied IAA had no additional effect on gravistimulated wheat pulvini. Of the hormones they tested, however, only IAA promoted growth in nongravistimulated tissue. Consistent with these findings, Brock and Kaufman (1988a) showed that low concentrations of IAA applied to gravistimulated oat pulvini produced merely an additive effect, whereas gravistimulus produced no additional bending in the presence of high concentrations of hormone. These findings are entirely in accord with the results shown in Figure 7, where 10 μM IAA enhanced cell wall incorporation by the same small percentage regardless of gravistimulus. Apparently, gravistimulus does not lead to increased sensitivity of pulvinar cells to exogenous IAA. In addition, neither Bridges and Wilkins (1973) nor Brock and Kaufman (1988a) found any effect of GA on vertical pulvini. These findings are in agreement with those reported in Figures 6C and 8C, where GA_3 had no significant effect on uptake or wall incorporation in vertical pulvini. Examination of wall synthesis in gravistimulated, GA_3-treated segments, however, was complicated by the disproportionate movement of label into the rapidly elongating internodal tissue, which apparently competed for substrate with the gravistimulated pulvinus. These results are intriguing because they point to new opportunities for studying source/sink relationships as modified by hormonal and tropic stimuli in an isolated plant part.

As reported previously with internodes dissected from the node and leaf sheath (Montague and Ikuma, 1975), GA_3 stimulated elongation and wall synthesis in the internodal tissue beginning between 1 and 2 h after hormone application (Fig. 10). FC mimicked the initial effect of GA_3 here with similar kinetics. The cell wall composition seemed to differ under the influence of this nonphysiological stimulus, however, with a greater proportion of TFA-extractable labeled material. FC also promoted internodal growth (elongation and fresh weight increase) whether the tissue remained attached to the rest of the segment or was isolated from it (Table I). The swelling (radial expansion) observed with FC, especially in isolated internodes, was also found by Metraux and Jones (1984) in GA-responsive lettuce hypocotyls. Toxic effects of FC were the probable cause of its ultimate inhibition of GA_3-induced elongation in oat stem segment internodes when supplied together with the hormone (Fig. 9). Although this inhibition of GA_3-induced growth is an unusual finding, it should be noted that FC is rarely studied over an extended time course of many hours. In one such study using auxin-sensitive *Avena* coleoptile sections, Cleland (1994) reported that FC-induced elongation ceased after 10 to 12 h, a time remarkably similar to that reported for *Avena* internodes in Figure 9. Even so, in contrast to the present results with oat, Stuart and Jones (1978) showed that FC actually promoted the GA_3-induced elongation of lettuce hypocotyl sections.

The stimulation of pulvinar cell wall synthesis by FC is similar to the findings of Brummell and Hall (1983) in elongating, auxin-responsive pea stem segments, in which the effect is apparently mediated at least in part by increased activity of glucan synthase (Ray, 1985, 1987). In the present work, FC stimulated incorporation predominately into the matrix (TFA soluble) component of the wall, whereas IAA and gravistimulus promoted incorporation proportionally into both fractions (Figs. 3–7). Thus, FC produced the same apparent difference in cell wall composition in both internodal and pulvinar tissues.

These results show that gravistimulus, IAA, FC, and GA_3 caused the same ultimate effects in their appropriate target tissue(s), i.e. growth (cell expansion) accompanied by enhanced cell wall synthesis, although the proportion of radioactivity found in TFA-soluble and TFA-insoluble components of newly synthesized wall material indicates that the wall composition may differ in the case of FC. The target tissue/stimulus interactions produced a distinctive pattern (summarized in Table II), which points to opportunities for further work. It should be possible to study, using this 10-mm *Avena* stem segment, those events in gene regulation and signal transduction that are specific to a particular tissue/stimulus combination, as opposed to those that are simply involved in cell elongation and/or cell wall synthesis per se, unrelated to a specific causal agent.

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